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Professor J. Lederberg,  
Department of Genetics,  
University of Wisconsin,  
Madison, 6.  
WISCONSIN, U.S.A.

Dear ~~Professor Lederberg~~ *John*,

Thank you for your several letters, including the copy of the ~~last~~ one. I am not really ready to reply yet as I have done nearly nothing about outline of paper, but some of the points which come up are relevant to it so I will make this a triangular letter to you and Norton.

- (i) Strains which I would be glad if you would send me are: the marked O strains SW 665 and 666, which I think would be good to use in micro-manip. experiments, when I start them: the various monospecific transformees of SW 543 (~~which~~ I agree they don't need detailed serological analysis, but the Colindale people, who have given me lots of sera, etc., would like to have them for possible use in making H sera): the non-b (or weak b) variants derived from SW 543 by F.A. abony or otherwise, Joan Taylor I think would like to look at them for us: your S. abony strain, as the only one I have available here is resistant to PLT 22.
- (ii) I will send you shortly the para. A.O strain and its apparent i derivative (have not yet had Joan Taylor's report on this but I think it is OK). I do not propose to do anything on possible linkage to the 543 locus, etc. so please go ahead with this. I will also send some of the other O forms I have got hold of here.
- (iii) Points to be completed to enable paper to be written.
  - (a) "Unmasking" of latent H antigens. I think this can only be done conclusively by use of F.A. from strain having no common H antigen with the transformed strain: I have completed this for all Joan Taylor's recent Group B "O" isolates, and will shortly check up on the SW O's so far as not already done. It would I

think be useful to be able to document the origin of the O's. SW 544 I know about (it is in N.C.T.C. 3 times over under different numbers, and I had it under 5 labels here, at first I thought we had a nice set of recurrences of the "slow" character but now all is explained). SW 543 is also fully covered. This leaves SW 541 only unaccounted for: do you have any details of its origin? 548 and 549 I take it were isolated as O forms, 545 I see is listed by Edwards as typhi-murium O, do you know whether this means it arose in laboratory as variant from an H?

- (b) Test of non-allelism on different S. t-m O strains: after eliminating duplicates I have 9 of these, (apart from chi-selected mutants) and I am doing a chess-board test to extend present fragmentary results.
- (c) Apparent allelism. I am setting up experiments to get several O forms from each of SW 592 and SW 593, by Chi selection, to see if I can get apparent recurrences: also I will test what are believed to be pairs of strains from same "epidemic" to see that they don't motilise each other: if this works it would be nearly a test of identity.
- (d) I don't know if present paper would be a good place for further documentation of idea that FA is phage-borne. If so identification of PLT 22 and PLT 7 in Boyd's scheme might go in here, and also competence of his strains as transducers. I enclose his report: he has ignored the filtrates and examined only the parent strains, which complicates things a bit because of the doubles. I have asked him for labelled filtrates as he suggests, also for anti-A 1 serum to check that PLT 22 is in A 1, etc. serological groups. I shall do nothing further in this direction I think, except that if the serum works I might try an FA-neutralisation test, which would confirm that FA is phage (or phage-bound).
- (e) Experiments to confirm or disprove idea that SW 573, the only S. t-m O which can't be motilised, is paralysed as well as O. Have not started this yet but it should be simple enough. I think one or two other experiments to test whether most strains can reverse the paralysis character also needed. This might be extended to "curly" also but I don't think this important.

*Trails* *flames*  
 (f) Traders and ~~places~~ (induced) should I think be mentioned but not gone into. Your failure to recover O's from ~~places~~ is interesting: on going through my notes I see that I recovered some spontaneous swarms, e.g. from SW 534 and 538 as having "spotty centres", i.e. probably "~~places~~", so a very general explanation is needed. I suggest following as purely speculative hypothesis: growth of H cells in micro-colonies at point of origin of swarms is due to (i) accidental trapping of some H cells in agar channels, (ii) a cell does not escape before 1 or 2 divisions, though many progeny will swim away some, not (iii) whether the residual cells form a visible colony will depend on how long growth continues after entrapment, (iv) entrapped cells near point of origin of swarm have a head start, so they only have time to give visible colonies before growth arrested everywhere by over-crowding. This I think covers both the decrease in colony size from centre to periphery of the ~~p flames~~, and apparent origin of some ~~flames~~ a few mms. from edge of O growth. Have just thought of this and made no tests.

(g) flagellar antigenic phase. This also I think should be mentioned but not gone into much. Some of my results conflict with yours, see below. I have experiments started to see if, as I expect, a recipient O cell is already in group or specific phase, unexpressed, and to check that phase of induced H cells is independent of phase of lysed donor cells. (All this in t-m or t-m experiments). Perhaps the paper should include your abony + t-m experiments with new phase combinations to exclude alternative allelic states as explanation of phase phenomenon.

I think that all, or maybe more than all, that's relevant to a paper at this point.

(iv) Influence of serum on transduction. I am much surprised by your finding that serum, added late, affects transduction of gal + trait. I would not be surprised if b anti-serum inhibited production of i swarms by SW 543 (though I have no evidence of it) since I presume the stable double transduction to have gone via an unstable state with foreign genes lying free: in this state cell might have antigens i or b or both, and in the latter cases motility might be inhibited, and if chromosomal location is delayed for several cell generations, the arrest of growth by over-crowding might precede, and so prevent it.

- (v) The b reactivity of 543 surprises me. I have not yet had Joan Taylor's report, but when I last saw her she had found nothing unexpected in antigenic analysis of the strains I sent, which included 543 and its spont. and induced H derivatives with b, i or l. I take it that the b-reactivity has been demonstrated only by agglutination; do you find it present at some time in all stocks of 543? If the b reactivity is regularly present in some environments it should be possible to demonstrate antigenicity and adsorbing capacity. I have a rabbit being immunised with SW 543 at the moment (in a class demonstration).
- (vi) I was glad to have explanation of unexpected monophasicness of SW 588, which I had noticed but not looked into. You say, SW 546 is the only strain whose 1, 2 antigens can be transformed to SW 543: I take it this is based on tests with FA from S. para. B, etc. as well as S. t-m. Do you know what reason Edwards had for calling SW 546 para B, rather than, e.g. stanley? Would you like me to send 546 to Felix to see if it phage-types as para. B?
- (vii) Thanks for the note on 30°- 37° differences in trail/swarm ratio: this might be related to Peggy Lieb's, etc. findings on temperature effect on pre-lysogenic state.
- (viii) I am impressed by your serial transductions done to exclude your hypothesis. I don't know on how much data N. based his generalisation that serial transduction is difficult. I would have expected that it was due to non-effective absorption, not failure to absorb, though the MS presumes the latter.
- (ix) As to terminology, I think the recipient cell which we study is transformed, transmuted (or transumed?) only, and that the new trait which it shows is induced: the latter term drops the trans suggestion, and so covers both cases, where the donor does or does not manifest the character. The phage might be said to carry in or across, transduce, transport, import (or transume) the postulated determinant, but not the character itself. I can't think of a good verb to abbreviate "a lysate of..... transforms....." or "induces..... in....." or a word to replace FA, though "lysate" would usually do. As the hypothesis is now fairly clear and simple I don't think the terms used matter provided they don't contradict the hypothesis by implication and are used consistently. "Transume" is the only word I can find which seems to mean either "carry across" or "transform", and I am not keen on it.
- (x) I don't think there is much logical reason for replacing "specific" names by code-numbers: the taxonomic units

defined by antigenic structure (and its range of demonstrable mutability) are of practical value, and theoretically valid, since other characters, e.g. pathogenicity, correlate with them. Whether to call them species ~~or~~ *sub-species* ~~variants~~ and to use names or antigen representations, are matters for arbitrary decision on practical grounds. Our "artificial" combinations do not deserve taxonomic recognition any more than do artificial hybrids which have not established themselves in a "natural" habitat.

- (xi) Having disposed of the oddments I get to the interesting part. Your November 2 letter, foot of page 2, says you find phase 2 FA has no phase 1 activity, on a diphasic H recipient or on 543. I have not tried H recipients, but on 543 I find PLT 22 lysates of LT 2 or of SL 46 (7 N.C.T.C. 73, S. t-m with relatively low rate of mutation of phase) have substantially the same activity on 543 whether the donor was lysed when in phase 1 or 2 (and the cultures were over 90% in stated phase I know, probably nearer 99% in case of SL 46): that is, all gave both b and i, and nothing else, (tested by plating <sup>with</sup> or without b serum). Also I always, both here and at Madison, got more b than i, usually about 3 to 1, you get the opposite ratio (your letter November 4, para. 5). The difference seems to be too consistent to be coincidental. I conclude there is some relevant difference in the way we set up the experiments. I use equal volumes of overnight broth culture of recipient and of phage, titre usually in range of  $3 \times 10^9$ , incubate at  $37^\circ$  for 30-60', and either plate directly, or if looking for doubles plate the resuspended deposit after spinning, on Edward's medium, incubate at  $37^\circ$  for 10-18 hours. Any obvious differences? A hypothesis to unify your and my results would be that the way you do it, mostly 'long' chromosome pieces replace their homologues, the way I do it mostly short pieces. Then in 543 experiments most of your cells receive in one piece the relevant H+ gene, the b gene and the gene(?) which "activates" it, anyway a phase determinant, but in my experiments most cells get only one gene, the H+ one, some get two, H+ and i and few if any get all three, so the phase 2 lysate is about as effective as phase 1. & I look forward to your reactions to this.

Incidentally how did you get e, n, x into 543? In its combinations this bunch seem to be a typical phase 2 antigen, so its as odd as the induced 1.2. phase.

- (xii) Finally, and with my apologies for excessive length, if you are still looking for quant. test for proportion of O's, have you tried making a pour plate of c 30 cells in Edward's motility medium, incubating at c  $23^\circ$  till colonies visible (about 2 days) then 2 or 3 hours at  $37^\circ$ , and look for non-

swarming colonies. It works more or less for phase determination, (only tried once so far and did not use quite enough serum) and if one used both phase sera in moderate concentration one might be able to use 100 or 20 cells perhaps.

(xiii) I enclose copies of Boyd's letter, and of my current stock list, which may be convenient for future reference.

Yours sincerely,

B.A.D. Stocker.

I hope you appreciate the typing, not done by me but by Spooner's secretary, who has had a little trouble with my writing at one or two points as you see. I have no news to speak of: I am still struggling with my fellowship report, not just over-due. I have recovered from my chagrin over your elections (I was betting on S. but really as well as metaphorically). Have just had a thing from the Internat. Genetics Congress people: I don't think them 'cheap' hotels v. cheap. How are you plans for Europe maturing? An interesting thing about PLT 7: have I asked Boyd's assistant (B. is in US) to look at the phage 7 I sent to see which B phage it contained, he has just phoned to say there is a B and an A phage which is odd: I will ask N. for details on how he stirred up the stream to make it self-lysing, & see if I can repeat it. (My positive transduction with Boyd's B4 may be wrong, I find my phage stock infected) My regards to all about your lab. Yrs Bruce